2616-Pos Board B602
Using Dynamic Force Spectroscopy to Study G-Quadruplex Disruption
Jen-Chien Chang, Michel de Messieres, Arthur La Porta.
Guanine-rich sequences in nucleic acids tend to fold into G-quadruplexes, in which guanines form stacked tetrads stabilized by hydrogen bonding and metallic ions. This structure is present in eukaryotic telomeres as well as the promoter region of genes, playing a regulatory role in various biological processes. Practically, it is also a target for drug delivery in cancer therapy. From a fundamental point of view, the kinetics of G-quadruplex needs to be studied in detail. To probe its structural stability, we apply optical tweezers and disrupt a single molecule of DNA G-quadruplex in vitro. Dynamic force spectroscopy is employed, in which the distribution of rupture forces for different force-loading rates are measured. By fitting the force distribution curve to a theoretical model, height and distance of the barrier in the energy landscape are extracted, providing an insight into the unfolding process of G-quadruplex.

2617-Pos Board B603
Full Reconstruction of a Vectorial Protein Folding Pathway by Afm and Smd: Insights Into the Co-Translational Folding of the Nascent-Polypeptide-Chain
Whasil Lee, Xiancheng Zeng, Huan-Xiang Zhou, Vann Bennett, Weitao Yang, Piotr Marszalek.
During co-translational folding, the nascent-polypeptide-chain (NPC) is extruded sequentially from the ribosome exit tunnel and under severe conformational constraints dictated by its 1D geometry. Here, we combine single-molecule atomic force spectroscopy and steered molecular dynamics simulations to examine protein folding in the presence of 1D constraints that are similar to those imposed on the NPC. The simulations exquisitely reproduced the experimental unfolding and refolding force extension relationships and led to the full reconstruction of the vectorial folding pathway of a large polypeptide, the 253-residue consensus ankyrin repeat protein, NI6C. We show that fully stretched and then relaxed NI6C starts folding by the formation of local secondary structures followed by the nucleation of three N-terminal repeats. This rate-limiting step is then followed by the vectorial and sequential folding of the remaining repeats. However, after partial unfolding, when allowed to refold, the C-terminal repeats successively regained structures without any nucleation step, by using the intact N terminal repeats as a template. These results suggest a pathway for the co-translational folding of repeat proteins and have implications for mechanotransduction.

2618-Pos Board B604
Force Spectroscopy of Add Adenine Riboswitch Aptamer Folding Reveals Multiple Intermediate and Misfolded States
Krishna P. Neupane, Hao Yu, Daniel A.N. Foster, Feng Wang, Michael T. Woodside.
Riboswitches regulate genes expression through ligand-induced conformational changes. The folding of the add adenine riboswitch was investigated by repeatedly unfolding and refolding single aptamer molecules held under tension with optical tweezers. Multiple partially-folded intermediate states were identified from force-extension curves and folding trajectories at constant force, and characterized by measuring the associated molecular contour length changes, kinetics, and energetics. These states were correlated to essential structural components and interactions in the aptamer in a hierarchical folding pathway. In particular, the transition state for unfolding the ligand-bound aptamer involved the disruption of triplex helix-junction near the adenine binding pocket. The action of this riboswitch as a translational regulator (1) is found to be determined by the thermodynamic properties of the riboswitch, not by its kinetic properties as for the similar pbuE adenine riboswitch (2). In addition to on-pathway intermediates, several off-pathway, ''misfolded'' states were also observed and characterized. These results extend our understanding of the mechanics of RNA structure formation, the effects of multiple folding pathways, and the relation between folding and function in riboswitches.
(1) Serganov, A., Yuan, Y.R., Pikovskaya, O., Polonskaia, A., Malinina, L., Phan, A.T., Hobartner, C., Micura, R., Breaker, R.R., and Patel, D.J. Structural Basis for Discriminative Regulation of Gene Expression by Adenine- and Guanine-Sensing mRNAs. Chem. Biol. 11: 1729-1741 (2004).
(2) Greenleaf, W.J., Frieda, K.L., Foster, D.A.N., Woodside, M.T. & Block, S.M. Direct Observation of Hierarchical Folding in Single Riboswitch Aptamers. Science 319, 630-633 (2008).

2619-Pos Board B605
Changes in Mechanical Properties Occur During Differentiation Within the Oligodendrocyte Lineage
Anna Jagielska, Adele Norman, Graeme Whyte, Robin J.M. Franklin, Jochen Guck, Krystyn J. Van Vliet.
Myelination, the process in which the myelin sheaths are formed around axons, is critical for the efficient transmission of nerve impulses and maintenance of axonal integrity in the central nervous system. Myelin sheaths are made by oligodendrocytes, which are derived from a neural stem cell called an oligodendrocyte progenitor cell (OPC). The mechanism of differentiation and causes of differentiation failure that limit oligodendrocyte regeneration in demyelinating diseases are incompletely understood. OPCs are subjected to mechanical strain, as well as chemical cues during their differentiation into a myelinating phenotype, especially when contacting growing axons during development. We hypothesize that mechanical strain plays a role in oligodendrocyte differentiation. Here, we characterize the viscoelastic properties of living OPCs and differentiated oligodendrocytes using atomic force microscope-enabled nanoindentation. Changes of cell compliance during the course of differentiation could alter the susceptibility of cells to external mechanical stress, as well as their adhesion and migration properties. Our results indicate that while populations of both cell types reveal a large variation of average cell stiffness, ranging from 0.5 to 20 kPa, the differentiated cell population contains a large subpopulation (65%) of significantly stiffer cells. These results indicate mechanical signatures within the oligodendrocyte lineage as a function of differentiation state, and provide a baseline for ongoing explorations of mechanically induced differentiation of this stem/progenitor cell.

2620-Pos Board B606
Electrostatic Effects of Multivalent Salts on SsDNA Elasticity
Dustin McIntosh, Omar A. Saleh.
Nucleic acids are highly-charged polyelectrolytes whose structure and function strongly depend on the concentration and type of salt ions in solution. We have created a simple experimental system for studying interactions between nucleic acids and salt ions, based on magnetic-tweezer measurements of the elasticity of single denatured ssDNA molecules in solutions with a known salt concentration. Using this system, we were able to reconcile single-molecule force-extension data with scaling theories of self-avoiding polymers, and we found that the Kuhn length of ssDNA scales with the Debye length in NaCl solutions (Saleh *et al.*, PRL **102**, 068301 (2009)). Here, we use the system to investigate interactions of ssDNA with multivalent salts. We find that, in divalent salt, ssDNA elasticity is qualitatively similar to that in monovalent salt, but with significant quantitative differences. Notably, at low ionic strength, ssDNA in divalent salt maintains the same low-force scaling behavior ('Pincus blob' regime) as seen in monovalent salts. However, there are differences in the elastic behavior at high forces ($>$ a few pN). In addition, analysis of the low-force scaling behavior indicates it requires ~100 fold smaller concentrations of divalent salt to condense ssDNA. We discuss the data in the context of electrostatic theories, including Debye-Huckel, as well as bulk experiments on similar systems.

2621-Pos Board B607
Nanomechanics of Collagen Type I Fibrils using Afm
Himanshu K. Verma, William G. Matthews.
By mass, collagen is the most abundant protein found in mammals. The fibril forming collagens self-organize, forming hierarchical structures from the molecular collagen building blocks. In particular, type I collagen provides the mechanical structure for such tissues as bone, dentin, ligament, tendon, cornea, as well as many soft tissue matrices. The wide range in mechanical properties evidenced by tissues provokes the question of how do they develop from essentially the same scaffolding material? To understand this process, the mechanics at the molecular, fibrillar, and whole tissue levels must be characterized fully and connected. It is at the fibrillar level the presented work was focused. Atomic force microscopy was used to perform force spectroscopy on individual type I collagen fibrils under varying solution conditions, including pH and ionic strength. Results from parallel studies at the molecular scale were used to interpret the data. The outcomes are of interest to the fields of cell differentiation and tissue engineering, among others.

2622-Pos Board B608
Probing the Viscoelasticity of Collagen Solutions with Optical-Tweezers-Based Microrheology
Marjan Shayegan, Nancy R. Forde.
Type I collagen is the major fibrillar protein of connective tissues and is well known for its broad range of functions throughout the body. Individual triple helical collagen molecules undergo self-assembly to form higher-order structures including fibrils and networks. Since this process is strongly dependent on temperature, pH and ionic strength, it is believed that electrostatic, hydrophobic and entropic interactions are the main forces which direct fibril formation.
Determining the mechanical behavior of collagen systems, from molecules in solution to a network of entangled chains, provides an understanding of the physical and chemical interactions between collagen chains that may contribute to fibril formation. To probe the micron-scale viscoelasticity of collagen solutions, we use optical tweezers and measure the local Brownian motion of an embedded probe particle. From high-bandwidth measurements (up to 100 kHz) of the particle's displacement, we obtain the local complex shear modulus of collagen solutions. In this study, we probe the concentration dependence of viscoelastic response and